EFFECT OF MODE OF RIPENING ON ETHYLENE BIOSYNTHESIS DURING RIPENING OF ONE DIPLOID BANANA FRUIT

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ABSTRACT

Ripening is the main physiological process that affects the behaviour of fruit quality traits. The progress of this process in banana fruit is different for fruit ripen or not on the tree and according to the treatment applied to the fruit after harvest. We investigated the effect of the mode of ripening on ethylene biosynthesis of IDN 110 (*M. acuminata*, AA) banana fruit ripen *in-planta (*in-Fruit*)* and *ex-plant* on air (air-Fruit) or after acetylene treatment (ace-Fruit). The levels of ACC and ethylene production and, those of ACC oxidase (*MA-ACO1* and *MA-ACO2*) and ACC Synthase (*MA-ACS1*) mRNA were examined in pulp tissue of each of these fruits. Our results showed that, from the harvesting point made at mature-green stage, the ripening speed of fruit was not correlated with ethylene production. Ace-Fruit took 10 days to reach over-ripe stage with a maximum of 22.6 µl.kg-1.h-1 of ethylene production, whereas Air- and in-Fruit took 27 and 33 days to reach over-ripe stage, respectively, and produced 11.5 and 29.6 µl.kg-1.h-1 of ethylene, respectively. During ripening, ACC accumulated differentially and, except for in-Fruit, this accumulation was positively correlated with that of *MA-ACS1* mRNA. No correlation was observed between ACC level and ethylene production. In all cases, the level of *MA-ACO1* mRNA was 100-fold more than that of *MA-ACO2,* and both of them increased during fruit ripening. In contrast to *MA-ACO2*, the pattern of *MA-ACO1* gene expression was correlated with that of ethylene production whatever the mode of ripening. The results herein suggested that i) the level of ethylene is not the unique factor that controls the speed of fruit ripening *in-planta*, ii) the level of ripening-ethylene production of the whole fruit is regulated at downstream step of ACC biosynthesis mediated by *MA-ACS1* gene and, in pulp tissue, the product of *MA-ACO2* might be involved in this regulation.

Keywords: *Musa*, gene cloning, gene expression, ACC synthase, ACC oxidase, quality, fruit

INTRODUCTION

- For fruit, quality traits including nutritional and organoleptic aspects can be considered as a source of biodiversity and increase the added value of the product and consequently the incomes for the producers

- Fruit Quality is a complex criteria (external and internal aspects as color, flavour, texture, aroma etc.) that is highly affected by both biotic and abiotic cues, some of them being antagonistic

- Elaboration of fruit quality traits involved number of complex physiological processes resulting from the coercitive action of different genes

INTRODUCTION

- As other fruits, the main quality traits of banana are set up during an important phase of its development namely ripening
- Banana fruit undergoes a ripening climacteric process characterized by a peak of respiration and a burst of an autocatalytic production of ethylene
- Concomitantly, there are molecular and biochemical changes leading to fruit ripening (peel degreening, aroma volatiles, sugar accumulation, fruit softening). Some of these changes are ethylene-dependant, ethylene-independent or both.

OBJECTIVES

- Characterize IDN 110, a dessert (AA) and diploid variety, in term of ethylene biosynthesis. IDN110 is a member of the core collection of banana varieties used as parents in breeding program

- Get more insights into the molecular mechanism that govern the ethylene biosynthesis process during ripening of IDN110, in order to identify the major (candidate) genes involved and related molecular marker

- The availability of characterized varieties parents of banana and, related marker of fruit quality traits are the key prerequisites for the implementation of the strategy (breeding and marker assisted selection) developed at CIRAD to improve of banana fruit quality.

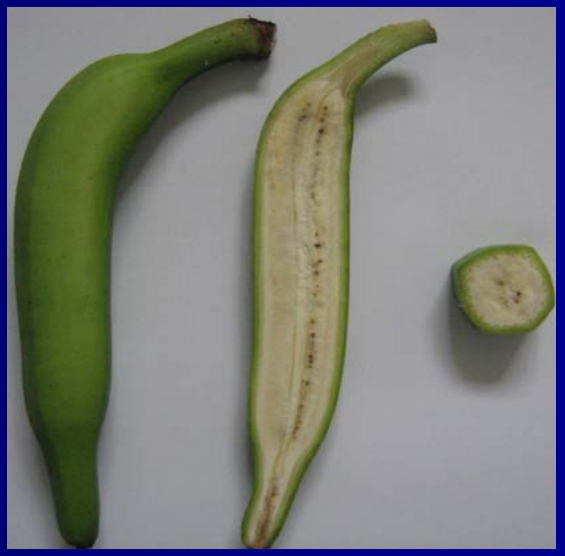
METERIAL

Growing – Harvesting

- MATERIAL - GROWING CONDITIONS :

IDN 110 (dessert cultivar, *Musa acuminata*, AA)

Guadeloupe ; altitude: 250 m; andosol; rainfall: 3500 mm / year



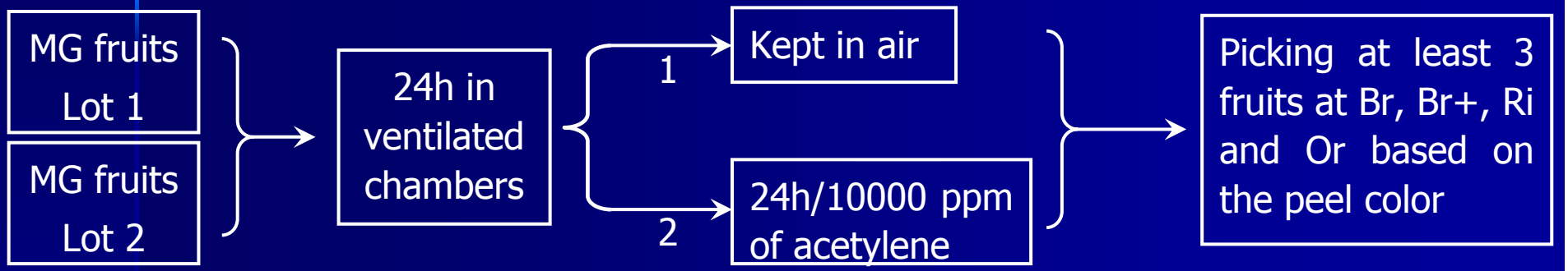
- HARVESTING *in-planta*:

Fruits were harvested at 6 developmental stages for all ripening conditions including IMG (immature green) and MG (mature Green), Br (breaker), Br+ (more breaker), Ri (ripe) and Or (over ripe). IMG and MG stages were harvested based on the heat units concept (Ganry and Meyer, 1975). While Br, Br+, Ri and Or stages were harvested based on the peel color

METHODOLOGY

Postharvest ripening - Physicochemichal and genes expression analyses

Postharvest ripening at 20°C (*ex-planta*):



Physico-chemical analyses:

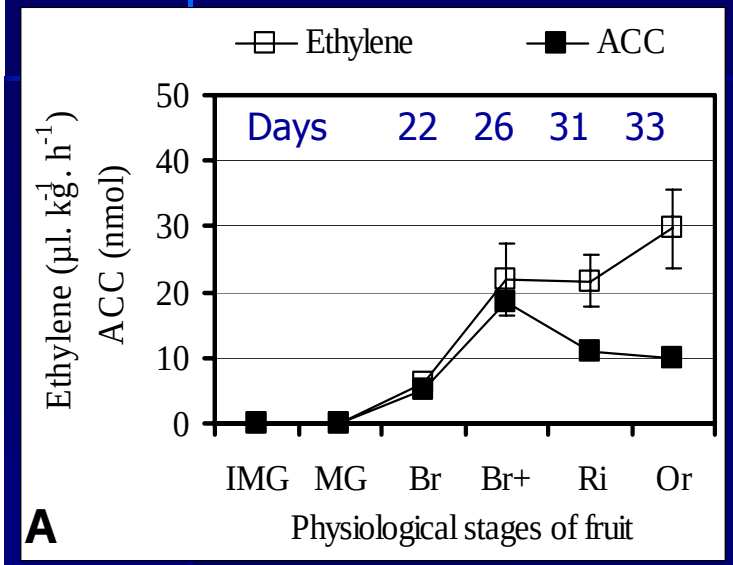
Measurement of ethylene production of the whole fruit and ACC content of the pulp tissue

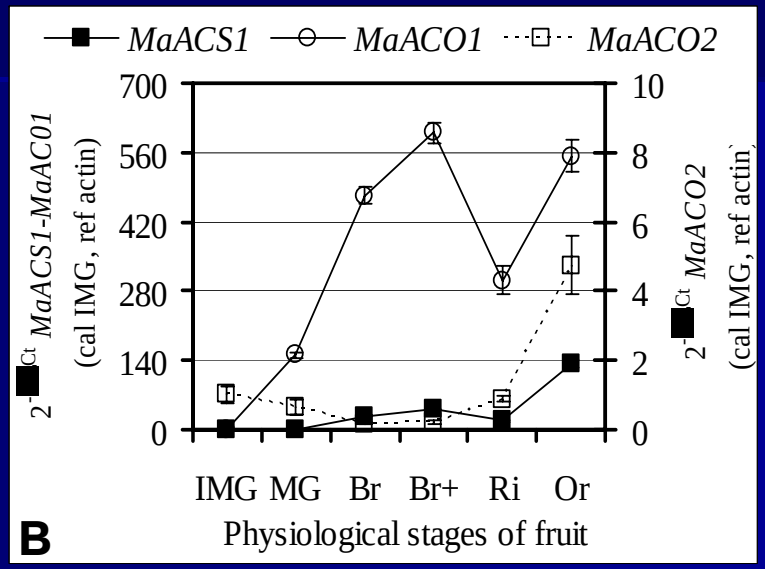
Gene expression analyses throughout Real-time Quantitative PCR:

- Two ACC oxidase genes, MA-ACO1 and MA-ACO2 (Inaba et al., 2008)
- One ripening and inducible ACC synthase gene, MA-ACS1 (Liu et al., 1999)
- mRNA level quantified using Double-Delta formula (Livak and Schmittgen 2001) with actin gene as reference and IMG stage as calibrator

RESULTS

Ethylene biosynthesis in-planta





Each data point is the mean of values obtained from at least three fruits originated from three replicate bunches. Vertical bars indicate standard deviation (S.D.). When no bar is shown, S.D. was smaller than the symbol. The numbers indicated within "ethylene-ACC" graphs represent the days taken by fruit, since IMG stage, to reach the corresponding physiological stage indicate at the abscise axe.

Ethylene production of the whole fruit and ACC content (A), ACC synthase and ACC oxidase genes expression (B) in pulp tissue of IDN 110 fruit ripening *in-planta*.

IMG =immature green; IM=mature green; Br=Breaker; Br+=more breaker; Ri=ripe.

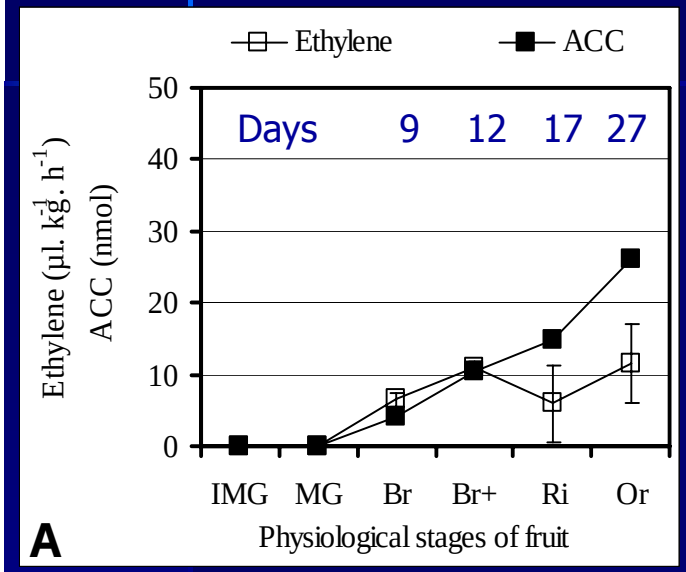
- Increase of C2H4 production throughout ripening with no fall in the rate as it was observed in triploid Cavendish banana.

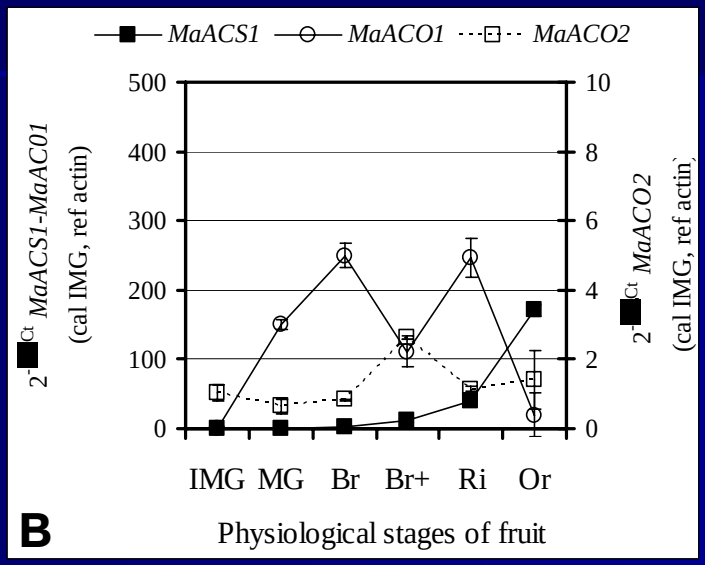
- No direct correlation between ACC content and the level of MaACS1 mRNA in pulp

- A direct correlation between ethylene production, MaACS1 and mainly MaACO2 mRNA accumulation in pulp

RESULTS

Ethylene biosynthesis ex-planta in air





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Ethylene production of the whole fruit and ACC content (A), ACC synthase and ACC oxidase genes expression (B) in pulp tissue of IDN 110 fruit ripening *ex-planta* in air. IMG =immature green; IM=mature green; Br=Breaker; Br+=more breaker; Ri=ripe.

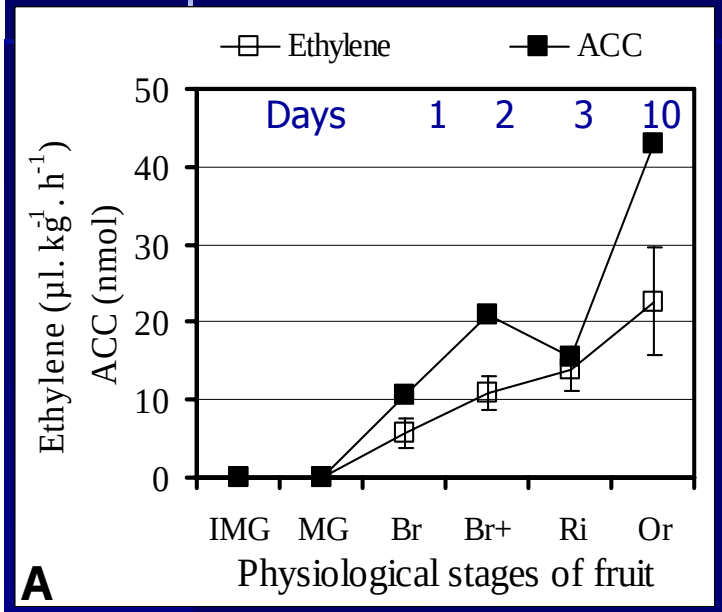
- Increase of C2H4 production throughout ripening with no fall in the rate as it was observed in triploid Cavendish banana.

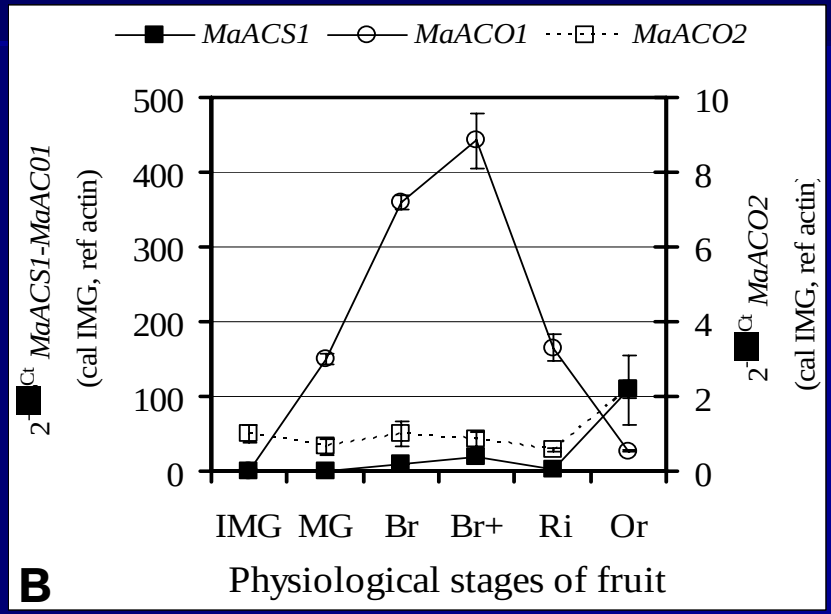
- Direct correlation between ACC content and, MaACS1 mRNA and the level of ethylene production

- A direct correlation between ethylene production and only MaACS1 mRNA accumulation in pulp

RESULTS

Ethylene biosynthesis ex-planta in air and after acetylene treatment





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Ethylene production of the whole fruit and ACC content (A), ACC synthase and ACC oxidase genes expression (B) in pulp tissue of IDN 110 fruit ripening *ex-planta* in air and after acetylene treatment. IMG =immature green; IM=mature green; Br=Breaker; Br+=more breaker; Ri=ripe.

- Increase of ethylene production throughout ripening with fall in the rate as it was observed in triploid Cavendish banana.

- Direct correlation between ethylene production of the whole fruit and ACC content, *MaACS1* mRNA and *MaACO2* mRNA accumulation in pulp

CONCLUSIONS

- IDN 110 fruit ripen slowly *in-planta* than *ex-planta*, and *ex-planta* in air than after acetylene treatment.

- No correlation was observed between the level ethylene production of the whole fruit and its ripening speed

- Whatever the ripening conditions, IDN110 displays a pattern of ethylene production different to that of triploid Cavendish banana, with no fall in the rate during the late ripening stage

CONCLUSIONS

- Difference between the time course of ethylene production of the whole fruit and, the pattern of ACC content and mRNA accumulations of ethylene biosynthesis genes

	C2H4	*ACC content*	*MaACS1*	*MaACO1 (highly expressed)*	*MaACO2*
In-Planta					
Air					
Acetylene					

PROSPECTS

- Characterize a large number of banana varieties in order to get more information the banana diversity in term of ethylene biosynthesis

- Examine, within these varieties, the relationship between the structural diversity of ethylene biosynthesis genes and ethylene biosynthesis in order to identify the putative marker usable in breeding to improve banana quality traits

- Investigate the mechanism of ethylene responsiveness process related with banana fruit ripening

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